*NATURE IN SINGAPORE* 2011 **4**: 157–161
Date of publication: 15 June 2011


# SPAWNING INDUCTION AND LARVAL DEVELOPMENT IN THE FLUTED GIANT CLAM, *TRIDACNA SQUAMOSA* (BIVALVIA: TRIDACNIDAE)

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## INTRODUCTION

Giant clams live in the warm shallow waters of coral reefs in the South Pacific and Indian Ocean (Rosewater, 1965; Carpenter & Niem, 1998). Their life history is bipartite, i.e., the adult is sedentary while the larvae are dispersed by currents (Scheltema, 1988). Larval dispersal, at least partly, explains their widespread distribution (Othman et al., 2010). Giant clams are highly fecund simultaneous hermaphrodites (but, unlike other hermaphroditic bivalves, they first spawn sperm then eggs) potentially releasing millions of gametes at each spawning (Lucas, 1994). Such fecundity may not translate into fertilization success and natural recruitment of juveniles is often very low (Gomez et al., 2000; Guest et al., 2008).

Giant clams have been cultured for decades, yet little research has described their reproductive development and life history. Previous studies have been concerned primarily with mariculture, especially nutrition, physiology, and growth rates (e.g., Jameson, 1976; Copland & Lucas, 1988; Klumpp & Lucas, 1994). However, early life information on the fluted giant clam (*Tridacna squamosa*) is limited (e.g., LaBarbera, 1974, 1975; Tan & Yasin, 2001). Here we attempt to fill this knowledge gap by describing the spawning, fertilization, and larval development of *Tridacna squamosa*.

## MATERIAL AND METHODS

**Spawning induction.** – The flow through aquarium system at the Tropical Marine Science Institute (TMSI) on Pulau Sakijang Bendera (= St. John's Island; 1°13´N, 103°50´E), Singapore, was used for spawning and experiments. Mature *Tridacna squamosa* brood stock (n = 30) was removed from a local reef (Raffles Lighthouse; 1°09′ N, 103°44′ E) and each clam was maintained in its own tank (length = 1.0 m, width = 1.0 m, depth = 0.7 m) in 1 µm-filtered, UV-treated seawater at ~29.5°C and 30‰ salinity. Six spawning trials were conducted between Feb.2007 and Sep.2007. Mature giant clams were injected with 2.0 ml of 20 µM concentration serotonin solution (crystalline 5-hydroxytryptamine, creatine sulfate complex, Sigma-Aldrich Pte Ltd, Product No. H7752-1G) into the gonads, via the mantle tissue beside the excurrent siphon region, to induce spawning (Braley, 1985). Each spawning trial comprised of four to five clams induced with serotonin solution and observed for spawning; clams were used only once for each conducted trial. When the clam released sperm (usually after ~30 mins), the sperm-suspension was collected in separate 10 l buckets and diluted to give a density of ~10,000 sperm $ml^{-1}$ (as determined from counts using a Neubauer haemocytometer). If eggs were released, the egg-suspension was collected in a fresh bucket and washed on a 22 µm plankton screen.

**Spawning of adult clams.** – During the period Feb.−Sep.2007, *Tridacna squamosa* spawned on four different occasions when induced with serotonin solution: 7 Mar., 18 Jul., 14 and 28 Aug.2007. There was only one observed *ex situ* spontaneous spawning on 24 Aug.2007, where only one clam spawned eggs. A 300 mm shell length adult was observed to produce ~660,000 eggs during a single spawning.

**Effect of egg-sperm ratios on fertilization success.** – The effect of four different egg-sperm ratios on fertilization success was determined using sperm and eggs collected as described above. The initial sperm concentration was ~10,000 sperm $ml^{-1}$ and the egg concentration was ~22 eggs $ml^{-1}$. To each of the eight 1000 ml beakers, ~11,000 eggs and different dilutions of sperm-suspension were added to give two replicates of each of the following egg-sperm ratios: 1:50, 1:100, 1:200 and 1:500. The total volume in each beaker was 800 ml (density of larvae = 13.75 $ml^{-1}$). After 3 hours and 24 hours, two 5 ml samples were collected using a pipette (after thorough agitation). At 3 hours, the numbers of fertilized eggs and non-fertilized eggs were counted. Fertilized eggs were identified by active cell division into a blastula cell mass, while undeveloped embryos were identified by no cell division. At 24 hours, the numbers of live and dead trochophores present were counted. All larvae types were counted on a Bogorov tray under a stereomicroscope.

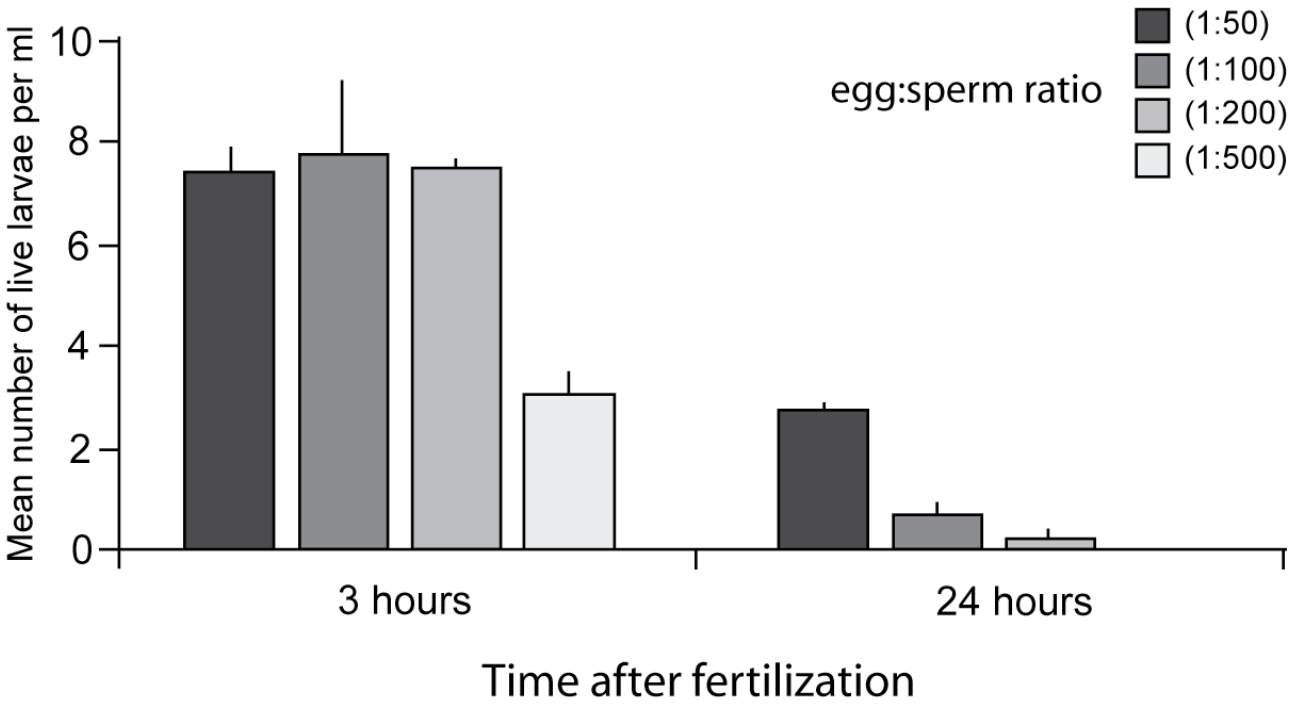


Fig. 1. Effect of egg-sperm ratios on the mean number of *Tridacna squamosa* embryos at 3 hours and trochophores at 24 hours. Error bars indicate standard error.

**Rearing of larvae.** – Developing embryos and trochophores (from 1:50 egg-sperm ratios) were stocked in "funnel" tanks, (diameter = 0.5 m, depth = 1.1 m) filled with 1 µm-filtered, UV-treated seawater. By day 3, larvae were fed with a mix of *Tetraselmis suecica* (CS–187) and *Chaetoceros mulleri* (CS–176) at cell density of 10,000 $ml^{-1}$. Only algae cells in the logarithmic phase of growth were used. Feeding continued from day 3 to day 6, except during zooxanthellae inoculation that was performed on day 5. Zooxanthellae were extracted from a piece of mantle biopsy by homogenising the tissue to release the cells. After 4–6 hours of feeding, daily water exchange using 1 µm-filtered, UV-treated seawater was performed to reduce the risk of bacterial infection or contamination. On day 7, clam larvae were introduced into the settlement tank as recommended by Ellis (1998).

## RESULTS AND DISCUSSION

Previous research on giant clams has focused on their growth and survival in mariculture (Beckvar, 1981; Heslinga et al., 1984). Even though giant clams release large amounts of gametes at each spawning; they experience high larval mortality before complete metamorphosis (Alcazar & Solis, 1986; Ellis, 1998). Part of this mortality may be due to non-optimal egg-sperm ratios, and this is examined here. We present a descriptive overview of fertilization ratios and early development of *Tridacna squamosa* embryos. Some later juvenile stages are also described.

**Effect of egg-sperm ratios on fertilization success.** – In this experiment, embryo survival (i.e., fertilization success) was almost similar for ratios 1:50, 1:100, and 1:200 but, after 24 hours, almost all trochophores reared at 1:200 and 1:500 egg-sperm ratios died (Fig. 1). After 3 hours, fertilization ratios at 1:50, 1:100, and 1:200 treatments had higher percentages of survival in embryos (53.8%, 56.4%, and 54.6%, respectively) than in the 1:500 (22.2%). For development to trochophores (sampled at 24 hours), the overall percentage of live larvae observed after 24 hours of development was 20% in the 1:50 compared to 5.09%, 1.82%, and 0.00% in 1:100, 1:200, and 1:500 treatments, respectively.

Fertilized eggs developed into the two- or four-cell stage within the first three hours of fertilization (Fig. 2a). Although the egg-sperm ratio was controlled, polyspermy was evident where eggs were penetrated by large numbers of sperm causing fertilized eggs to cease cell division or embryos were malformed (Fig. 2b). This phenomenon has been reported previously in giant clams (Braley, 1992) and this condition can cause cell death or abnormal embryonic development (Levitan, 2006). Excess sperm in the water column can also contribute to bacterial fouling and reduction of oxygen levels (Alcazar & Solis, 1986; Oliver & Babcock, 1992), providing an unhealthy environment for larvae survival. On the coral reefs, the number of giant clam sperm would normally be greatly diluted in the surrounding water — reducing the likelihood of polyspermy. In addition, spawning of sperm first followed by the release of female gametes may be an adaptation to ensure that competition between sperm (quality) for the eggs (limiting factor) (Munro et al., 1983; Michiels, 1998).

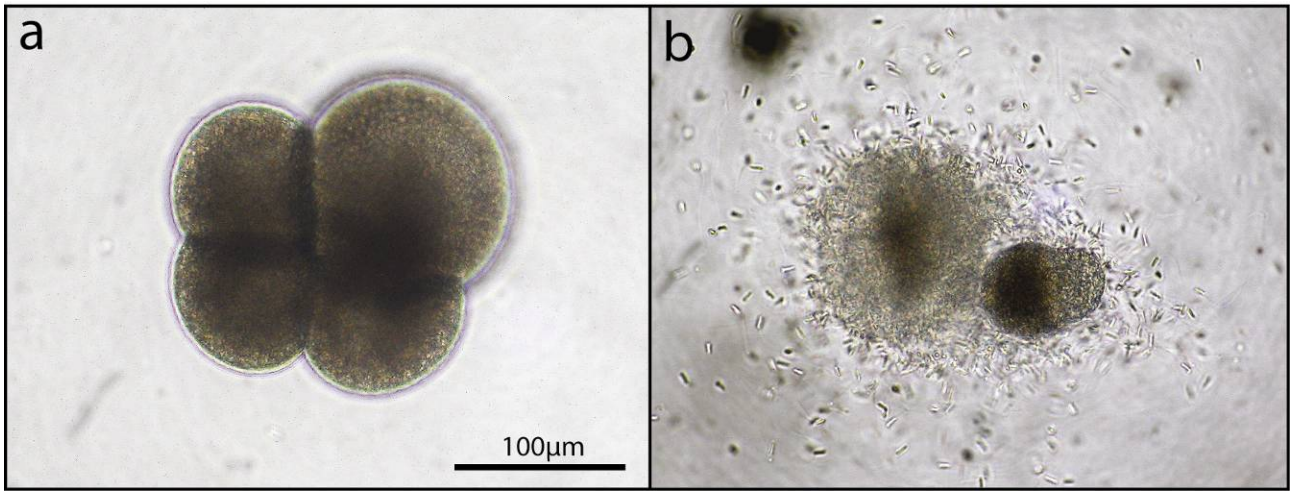


Fig. 2. Embryos were produced from spawning on 14 August 2007, 1600 hours. Unequal four-cell stage of *Tridacna squamosa* embryo showing three smaller and one larger blastomere (a); *Tridacna squamosa eggs* surrounded by a large number of sperm resulting in polyspermy (b).

When rearing giant clams ex situ, conditions need to be controlled to enhance larval development. The effects of sperm concentration affected the overall survivorship in trochophores suggesting that, of the tested ratios, 1:50 is the most suitable fertilization ratio as it resulted in the highest mean number of live *Tridacna squamosa* larvae overall (i.e., embryos and trochophores). In other molluscs, such as surf clams, *Spisula solidissima* (Dillwyn, 1817), and mussels, *Mytilus galloprovincialis* Lamarck, 1819, successful fertilization has been reported for egg-sperm ratios of between 1:50 and 1:100 (Dufresne-Dubé et al., 1983; Clotteau & Dubé, 1993).

**Larval development.** – Larvae produced using 1:50 egg-sperm ratio showed healthy cell divisions after approximately 3–4 hours. Trochophores were first observed swimming on their axis after 24 hours (Fig. 3a) and fully developed straight-hinge D-veliger larvae were first observed approximately 5 hours after trochophores (Fig. 3b). These veligers were actively swimming and feeding within the water column (Fig. 3b). Higher mortality occurred after the introduction of feed (including zooxanthellae inoculation); bacterial proliferation was observed, and dead veligers exuded internal granular material (Fig. 4). By day 5–6, veligers became less active (i.e., less swimming) and were observed to rest on the substratum (Fig. 3c). Seven-day old larvae began to crawl along the substratum using their ciliated foot (Fig. 3d), and settled onto the substrate by the eighth day (Neo et al., 2009). Further development of juvenile *Tridacna squamosa*: from translucent shell (Fig. 5a), to fully opaque shell development after 58 days (Fig. 5b), and finally pigmentation and scute development observed after 64 days (Figs. 5c, 5d).

## ACKNOWLEDGEMENTS

Many thanks go to Huang Danwei, Loke Chok Kang, James Guest, members of the Marine Biology Laboratory, the crew of the Mudskipper and staff of the Tropical Marine Science Institute. This research is supported by Singapore's Ministry of Education's ARF Tier 1 funding: grant number R-154-000-414-133.

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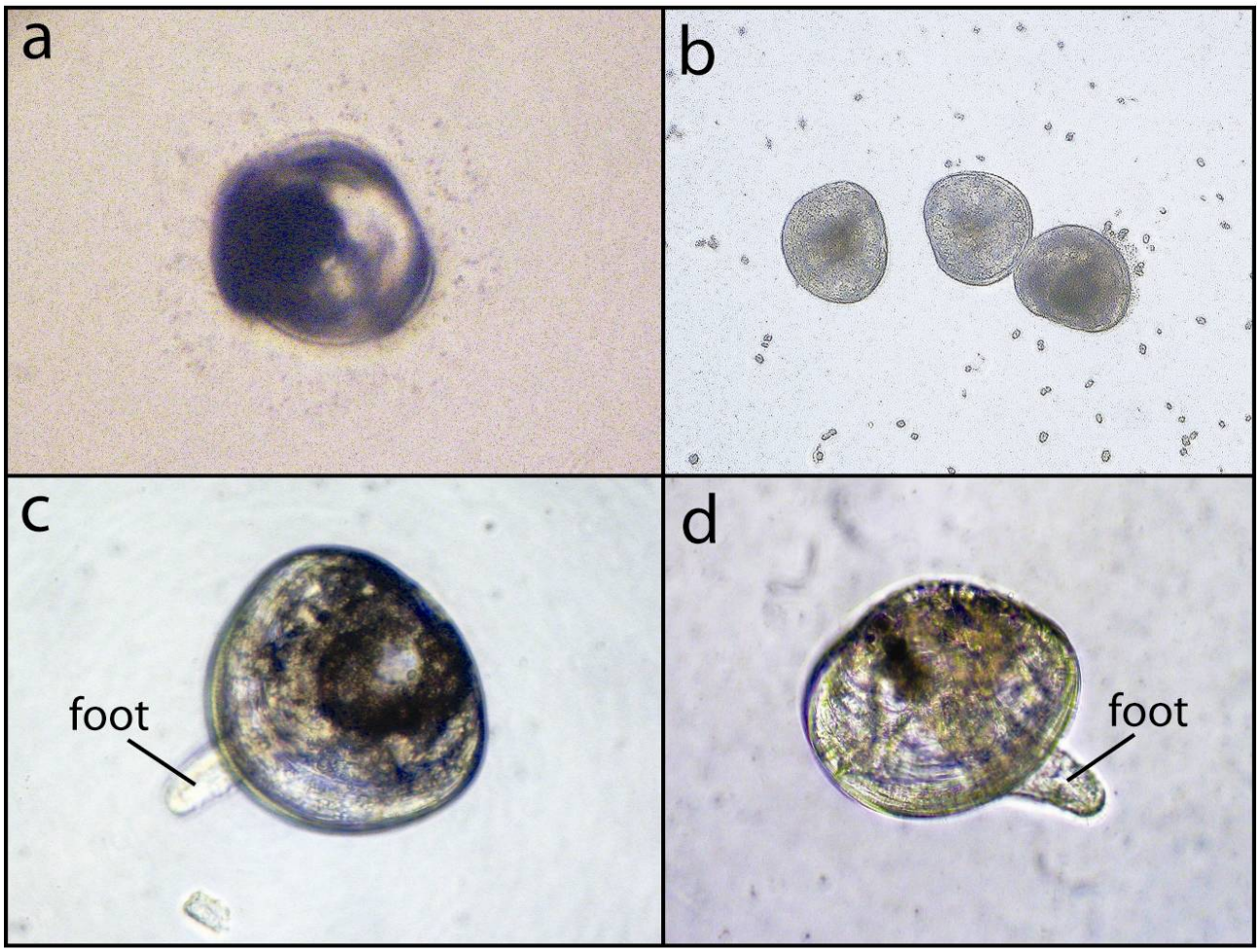


Fig. 3. Embryonic development in *Tridacna squamosa*. 1-day old trochophore spinning in the water column (shell length; SL = 100 µm) (a); 2-day old straight-hinge veliger feeding on microalgae (SL = 150 µm) (b); 7-day old pediveliger showing foot extension (SL = 220 µm) (c); 18-day old juvenile (SL = 600 µm) (d).

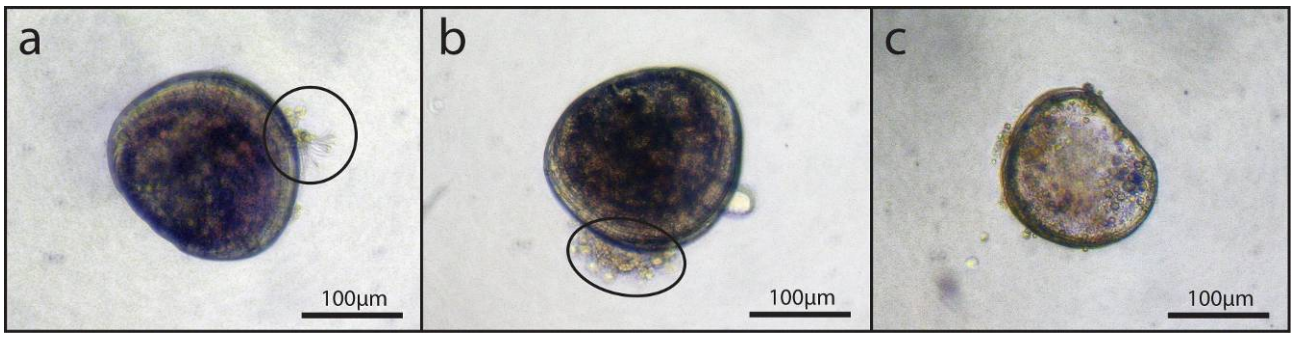


Fig. 4. Signs of infection and mortality in five-day old *Tridacna squamosa* veligers: Filamentous bacteria (circled) (a); internal granular material (circled) burst outside of prodissoconch (b); The empty mantle cavity of a dead veliger (c).

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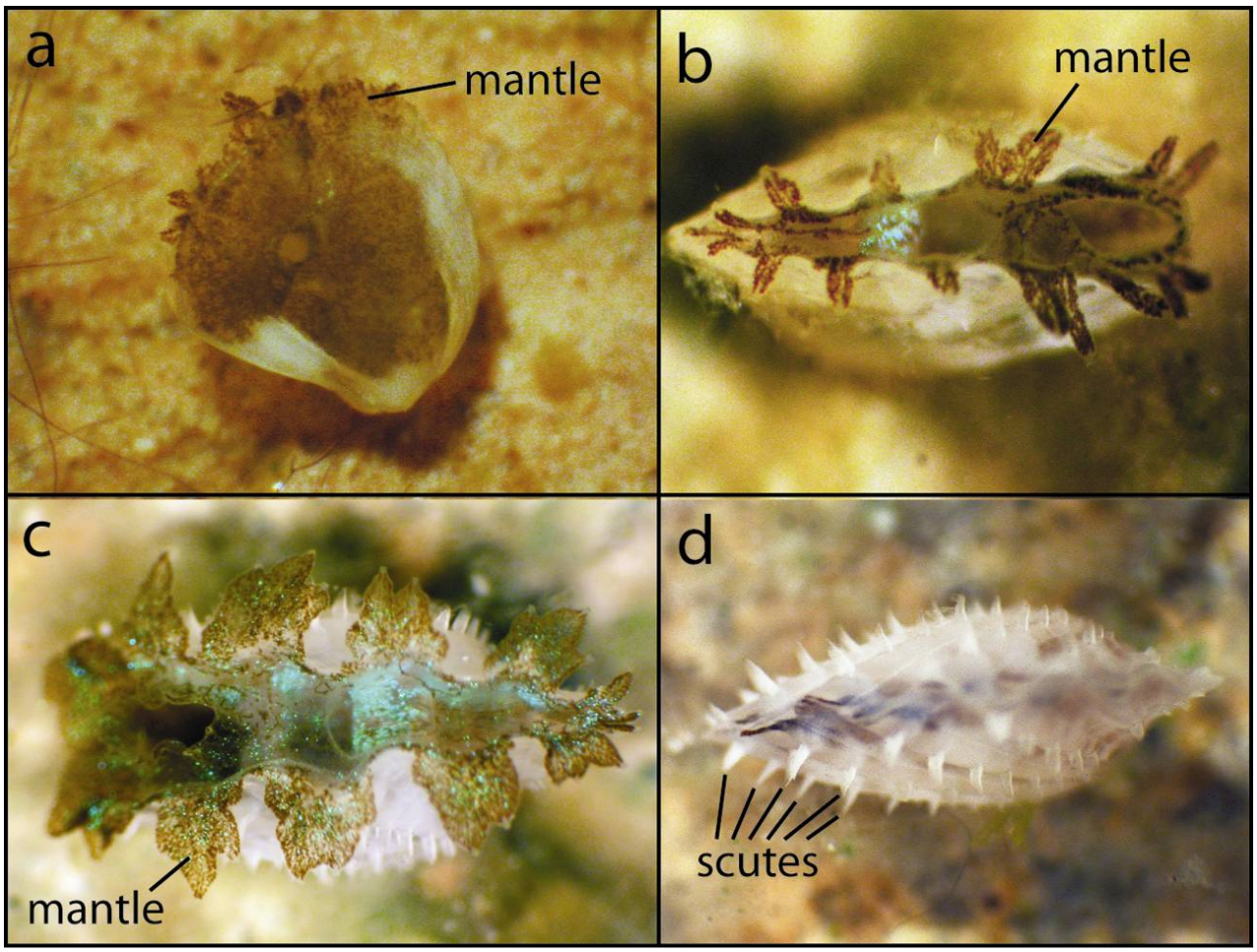


Fig. 5. Morphogenesis of juvenile *Tridacna squamosa*: 1-month old at shell length (SL) = 1200 µm (a); 2-month old at SL = 2000 µm exhibiting pigmented mantle (b); 2.5-month old at SL = 6000 µm exhibiting pigmented mantle (c); juvenile outer shells exhibiting scutes (fingernail-like projections) (d).

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